ORIGINAL ARTICLE

Mutations in the hereditary haemochromatosis gene HFE in professional endurance athletes

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Br J Sports Med 2004;**38**:418–421. doi: 10.1136/bjsm.2002.003921

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Accepted 20 May 2003

Background: Hereditary haemochromatosis, a disease that affects iron metabolism, progresses with a greater or lesser tendency to induce iron overload, possibly leading to severe organ dysfunction. Most elite endurance athletes take iron supplements during their active sporting life, which could aggravate this condition.
Objective: To determine the prevalence and discuss potential clinical implications of mutations of HFE (the gene responsible for hereditary haemochromatosis) in endurance athletes.
Methods: Basal concentrations of iron, ferritin, and transferrin and transferrin saturation were determined in the period before competition in 65 highly trained athletes. Possible mutations in the HFE gene were evaluated in each subject by extracting genomic DNA from peripheral blood. The restriction enzymes *Sna*BI and *Bcl*I were used to detect the mutations 845G→A (C282Y) and 187C→G (H63D).
Results: Our findings indicate a high prevalence of HFE gene mutations in this population (49.2%) compared with sedentary controls (33.5%). No association was detected in the athletes between mutations and blood iron markers.
Conclusions: The findings support the need to assess regularly iron stores in elite endurance athletes.

Hereditary haemochromatosis is an autosomal recessive disease in which the body's iron stores are increased, with serious negative effects on the function of several organs (liver cirrhosis, diabetes mellitus, heart failure).[1–3] The clinical consequences of iron overload in these patients can be prevented by early diagnosis and appropriate treatment.[4 5]

The HFE gene plays a major role in hereditary haemochromatosis. It occurs in the short arm of chromosome 6.[6] Most patients with manifest hereditary haemochromatosis are homozygous for the C282Y mutation, and a small proportion are heterozygous for both the C282Y and H63D mutation of the HFE gene.[7] Only a few affected cases show C282Y heterozygosity or H63D homozygosity. Subjects with these two genotypes generally display fewer markers of iron overload than C282Y homozygotes.[8] Although these patients have a very low or no risk of developing cirrhosis or other complications, some authors suggest that they should be treated similarly to C282Y homozygotes.[9] Moreover, despite the compound C282Y/H63D heterozygous defect expressing the disease with low penetrance (0.44–1.5%),[6 10 11] it has been identified as an independent risk factor of death from cardiovascular causes or myocardial infarction.[12 13]

There is considerable evidence to suggest that mutations in the HFE gene, even H63D heterozygosity, affect blood iron indices, and subjects with one or more mutations show higher blood iron concentrations and transferrin saturation than subjects without mutations.[14] Deugnier *et al*[15] recently explored factors that induce the increased iron reserves observed in elite cyclists, and suggested that increased serum ferritin was mainly related to the excessive iron supplements taken by these athletes rather than to mutations in the HFE gene (C282Y and H63D).

Despite the tremendous advances made in the area of genetic mutations in disease since 1996,[6 16] genetic evaluations such as screening the general population are not recommended.[17] Phenotypic analysis based on transferrin saturation still seems to be the best and most economical diagnostic method to either preclude or warrant a more in depth analysis of the disease in a particular subject.[18 19] Thus, if transferrin saturation is >45%, a genetic analysis of HFE mutations and serum ferritin determination would be called for, to better estimate the subject's iron stores.

This study was designed to evaluate the prevalence of HFE mutations in elite endurance athletes. The results were compared with those of a control group matched by region of origin.

MATERIALS AND METHODS

Subjects

Sixty five elite, male athletes (50 professional road cyclists and 15 Olympic class endurance runners) from Spain were enrolled in the study. Written consent was obtained from each subject according to the guidelines of the Universidad Complutense, Madrid.

The mean (SD) age, height, mass, and maximum oxygen consumption (V_{O_2MAX}) of the athletes were: 26 (3) years, 178 (5) cm, 66.7 (6.1) kg, and 71.8 (7) ml/kg/min respectively. The subjects were previously confirmed to be healthy by a medical examination including electrocardiography and cardiac ultrasonography. No subject had a familial or personal history of endocrine or metabolic disease. No exogenous substances had ever been detected in anti-doping checks performed in the subjects by the corresponding official organisations. Most (90%) of the athletes took iron supplements (not including the C282Y/H63D heterozygous subject (see the Results section)) at a mean dose of 105 mg Fe on alternate days for at least six months of the year. Doses and treatment regimens were similar in each subject.

A control group (n = 134) composed of random sedentary men from Spain also entered the study.

Measurement of blood iron markers in the athletes

Fasting blood samples were collected from all the athletes after at least three rest days during the period before competition. When available, serum ferritin concentrations

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(determined 12 and 6 months before the study) were recorded.

Serum iron concentrations were measured using a standard colorimetric method (Roche/Hitachi 714; Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Transferrin concentration was determined by rate immunoturbidimetry (OSAX anti-serum for the Behring nephelometer; Dade Behring Marburg GmbH, Marburg, Germany). Serum transferrin saturation was calculated from these data as follows:

Transferrin saturation (%) = (serum iron concentration (mol/l)/(2 × transferrin concentration (mol/l))) × 100.

Serum ferritin concentrations were measured by chemiluminescence immunoassay (N-latex ferritin kit; Dade Behring Marburg GmbH). The coefficients of interassay and intra-assay variability averaged 1.2% and 1.8% for serum iron, 2.3% and 2.7% for transferrin concentration, and 1.2–3.1% and 1.0–4.6% for serum ferritin.

Serum ranges considered normal were 13–32 µmol/l for iron, 24–336 g/l for transferrin, 24–45% for transferrin saturation, and 20–300 µg/l for ferritin.[18]

Study of C282Y and H63D mutations in all subjects

Genomic DNA was extracted from peripheral blood using a standard phenol/chloroform procedure followed by alcohol precipitation. DNA amplification was performed using polymerase chain reaction (PCR) with specific primers for the two HFE gene mutations as described previously.[10] The PCR conditions for both mutations were as follows: initial denaturation at 95℃ for five minutes; 35 cycles at 95℃ for one minute, 55℃ for 45 seconds, 72℃ for one minute, and a final extension at 72℃ for five minutes. The PCR products were then subjected to enzymic digestion for two hours, with restriction endonucleases cleaving the DNA at specific points such that the presence or absence of the mutations could be detected. The restriction enzymes used were *Sna*BI for the 845G→A (C282Y) mutation and *Bcl*I for the 187C→G (H63D) mutation. The digested fragments were visualised by electrophoresis on 2% agarose gels stained with ethidium bromide (fig 1).

Statistical analysis

All variables were tested for normality. We compared the distribution of HFE genotypes in both athlete and control groups with the Fisher exact test. Ferritin data for athletes were log transformed to normalise the distribution before analysis. Differences between genotypes in the athlete group were identified by one way analysis of variance. In this group, the paired *t* test was used to compare the variables according to the presence or lack of mutations. Correlation between paired quantitative data was assessed by the Spearman test. All statistical analyses were performed using SPSS 9.0 software for Windows. The level of significance was set at 0.05.

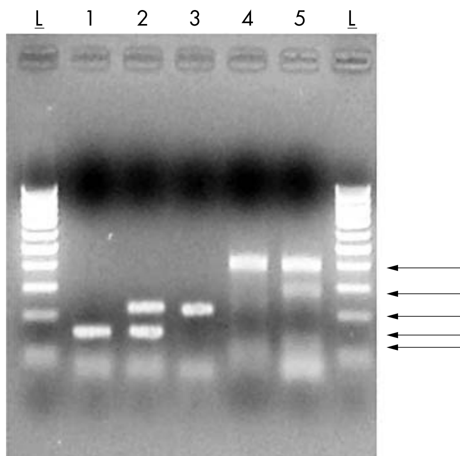

Figure 1 Agarose gel (2%) stained with ethidium bromide. Arrows (from top to bottom) indicate the fragments generated after digestion with *Bcl*I and *Sna*BI: 420, 300, 220, 160, and 120 pb. The samples applied were: lane L, molecular mass markers (ladder 100); lane 1, H63D (−/−); lane 2, H63D (−/+); lane 3, H63D (+/+); lane 4, C282Y (−/−); lane 5, C282Y (−/+).

RESULTS

Table 1 shows the prevalence of the different HFE gene mutations in the two groups. The proportion of subjects without a HFE gene mutation was significantly higher in the control group than in the athlete group (66.5% *v* 50.8%; p = 0.03). H63D heterozygosity occurred in 41.5% of the athletes and 24.6% of the controls (p = 0.01). No other significant difference was found between groups. No homozygote for the C282Y mutation was detected in athletes or controls.

Table 2 shows biometric variables, maximum aerobic capacity, and blood iron markers in subgroups of athletes established according to the type of HFE mutation carried. No significant differences in any of the variables (p>0.05) were detected among subgroups.

The different variables were also compared in the athlete group in terms of the presence or absence of mutations (table 3). No differences were observed among the subgroups.

DISCUSSION

The most important finding of our study was the high proportion of endurance athletes with a mutation in the HFE gene (49.2%), 29 (44.6%) of whom carried an H63D mutation and three (4.6%) a C282Y mutation. The prevalence of H63D heterozygosity was significantly higher (p = 0.01) than in controls (41.5% *v* 24.6%). Our results are in agreement with those of previous research showing that in the general population of Spain the prevalence of the H63D mutation ranges from 16%[20] to 30.4%,[21] and C282Y mutations occur in 2%[20] to 4.4% depending in both cases on the geographical region.[22]

Thus, the prevalence observed here (especially for H63D) is much higher than that previously reported in non-athletic subjects, yet is similar to rates recently observed by Deugnier *et al*,[15] who also warned of a higher prevalence of H63D mutations in French cyclists compared with healthy controls.[15] It remains to be seen if these mutations afford any metabolic advantage to these athletes during exertion. We detected no significant differences in V_{O_2MAX} between subjects with or without the mutation, thus we cannot confirm this hypothesis.

Although there appeared to be no relation in the athlete group between the presence and absence of mutation with respect to the blood iron markers, it is observed that 61.1% of

Table 1 Percentage distribution of HFE genotypes in elite endurance athletes and sedentary controls

Genotype	Athletes (n = 65)	Controls (n = 134)	p Value
wt/wt	50.8	66.5	0.03
C282Y/wt	3.1	4.5	NS
H63D/wt	41.5	24.6	0.01
C282Y/H63D	1.5	0.7	NS
H63D/H63D	3.1	3.7	NS

wt/wt, Wild-types; C282Y/wt, heterozygotes for the C282Y mutation; H63D/wt, heretozygotes for the H63D mutation; C282Y/H63D, compound heterozygotes; H63D/H63D, homozygotes for the H63D mutation; NS, not significant.

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Table 2 Biometric variables, maximum aerobic capacity, and blood iron markers in athletes (n = 65) grouped by HFE genotypes

	wt/wt	C282Y/wt	H63D/wt	C282Y/H63D	H63D/H63D	Normal ranges
Number	33	2	27	1	2	
Age (years)	26 (3)	28 (2)	26 (4)	27	27 (1)	
Height (cm)	178 (6)	175 (2)	178 (6)	183	172 (3)	
Mass (kg)	67.4 (5.2)	61.0 (0.5)	67.1 (6.6)	70	55.5 (2.1)	
VO_2MAX (ml/kg/min)	70.8 (6.8)	75.6 (0.9)	72.3 (6.6)	81.2		
Fe (µmol/l)	19.3 (5.7)	14.1 (0.3)	19.0 (6.4)	22.2	19.9 (9.9)	13–32
Ferritin (µg/l)	224.7 (153.2)	210.0 (141.4)	252.4 (148.9)	374	471.0 (80.6)	20–300
Transferrin (g/l)	232.4 (26.8)	174.0	224.4 (23.8)	174.5		24–336
TSI (%)	35.8 (8.0)	35.1	35.1 (12.1)	55.9		24–45

Values are mean (SD). No significant differences in any of the variables ($p>0.05$) were detected among the athlete subgroups.
wt/wt, Wild-types; C282Y/wt, heterozygotes for the C282Y mutation; H63D/wt, heretozygotes for the H63D mutation; C282Y/H63D, compound heterozygotes; H63D/H63D, homozygotes for the H63D mutation; VO_2MAX, maximum aerobic capacity; Fe, iron; TSI, transferrin saturation index.

Table 3 Comparison of variables according to the presence or absence of HFE gene mutations

	Age (years)	Height (cm)	Mass (kg)	VO_2MAX (ml/kg/min)	Fe (µmol/l)	Ferritin (µg/l)	Transferrin (g/l)	TSI (%)
HFE mutation	26 (3)	178 (6)	66.1 (6.9)	72.8 (6.5)	18.8 (6.3)	267.2 (151.3)	218.8 (27.6)	36.2 (12.4)
No HFE mutation	26 (3)	178 (6)	67.4 (5.2)	70.8 (6.8)	19.3 (5.7)	224.7 (153.2)	232.4 (26.8)	36.1 (10.8)
Normal ranges					13–32	20–300	24–336	24–45

Values are mean (SD). No significant differences in any of the variables ($p>0.05$) were detected among the athlete subgroups.
Fe, Iron; TSI, transferrin saturation index.

Take home message

The prevalence of mutations in the HFE gene (responsible for hereditary haemochromatosis, a disease in which the body's iron stores are increased) seems to be high among elite endurance athletes (about 49%). As most elite endurance athletes take iron supplements, regular assessment of their iron reserves is recommended to prevent iatrogenic iron overload.

athletes with high ferritin concentrations had a mutated HFE gene. It is known that long term exposure to small amounts of iron can lead to atherogenesis and ischaemia/reperfusion damage caused by free radical formation.[23] Even minimally increased iron deposits in people heterozygous for the mutations could be detrimental and enhance the risk of cardiovascular disease.[24] Thus, taken together, our findings and those of Deugnier *et al*[15] suggest the need to regularly assess iron status—that is, ferritin concentrations and transferrin saturation—in elite endurance athletes to prevent iatrogenic iron overload in this population.

In conclusion, the prevalence of HFE gene mutations is high among elite endurance athletes (runners and professional cyclists). Regular determination of their iron stores is thus recommended.

ACKNOWLEDGEMENTS

We thank Ana Burton for translation of the manuscript.

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Upper airway obstruction masquerading as exercise induced bronchospasm in an elite road cyclist

K E Fallon

This case concerns an elite road cyclist who complained of occasional dyspnoea and inspiratory difficulty during intense exercise. Clinical examination was normal and the final diagnosis was vocal cord dysfunction, a paradoxical closure of the vocal cords during inspiration which is highly associated with inspiratory stridor at high rates of ventilation. Awareness by the sports physician of this not uncommon condition is important to avoid misdiagnosis.

(*Br J Sports Med* 2004;**38**:e9) http://bjsm.bmjjournals.com/cgi/content/full/38/4/e9

Cardiovascular stress on an elite basketball referee during neonatal competition

A S Leicht

This case report examined the cardiovascular stress imposed on an experienced elite basketball referee during national competition. The average heart rate was similar for all matches, approximated 73% of maximum heart rate, and was experienced for most (>63%) of the match. Similar relative exercise intensity was demonstrated regardless of match play (men's *v* women's) and officiating type (two *v* three-referee). Further study is needed to document the physiological characteristics of elite basketball referees for greater performance.

(*Br J Sports Med* 2004;**38**:e10) http://bjsm.bmjjournals.com/cgi/content/full/38/4/e10

An unusual presentation of immersion foot

D M Macgregor

We report a case of "green foot" in a child with a plaster cast applied for a fractured metatarsal who subsequently re-presented with circulatory compromise. The foot was green and smelly and profuse *Pseudomonas aeruginosa* was cultured. The infection cleared with simple exposure to air. Perhaps this diagnosis should be considered in patients presenting with circulatory compromise in a cast as severe infection can result in amputation.

(*Br J Sports Med* 2004;**38**:e11) http://bjsm.bmjjournals.com/cgi/content/full/38/4/e11

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